A2

3. (amended) The method of claim 1 wherein the explant is a hypocotyl having a cut end below the cotyledon.

A3

8. (amended) The method of claim 4 wherein transformed roots are initiated in the hypocotyl by placing the end of the hypocotyl contacted with the *Agrobacterium rhizogenes* in a media containing ¼ strength Murashige and Skoog media.

A4

11. (amended) The method of claim 10 wherein the concentration of kanamycin in the media is no more than 50 mg/L.

REMARKS

Reconsideration of the application in view of the amended claims and the following remarks is respectfully requested.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1-11 under 35 U.S.C. § 112, second paragraph on various grounds. Applicants have addressed this rejection by way of a clarifying amendment to the applicable claims and present the following remarks. Regarding the use of the word "explant" in claim 1, it is submitted that this term is commonly used and known by one of ordinary skill in the art and the term is being used in a manner consistent with such common knowledge in the application and claims. Similarly, the use of the term "hypocotyl" is a term commonly used and known by one of ordinary skill in the art and the term is being used in a manner consistent with such common knowledge in the application and claims. A copy of definitions from Webster's New Collegiate Dictionary are submitted herewith as further evidence of the standard use and meaning of these terms. Claims 3, 8 and 11 have been clarified to remove any potential ambiguity.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 1-11 under 35 U.S.C. § 112, first paragraph as requiring a selectable marker step. Applicant's respectfully traverse and request reconsideration. Contrary to the position of the Examiner, a selectable marker/selection step is not required/preferred in the present invention. The elegance of the invention described and claimed herein is that by employing the method of the invention, one can rapidly and efficiently obtain a "chimeric" plant having transgenic root tissue and wild-type shoots, leaves etc enabling one to perform studies on the nucleic acid being expressed from the transformed root tissue. The transformation efficiencies of the *Agrobacterium rhizigenes* in the roots can be between 40-

70% (see specification pages 7, 10 and 12) without selection which is sufficient to employ rapid screening assays on the nucleic acid of interest. It is not in all situations that one needs or desires a stably transformed plant that expresses the nucleic acid of interest in all tissues. This invention addresses that need. As stated in the specification at least on page 10, a selectable marker and selection step may be included to increase the transformation efficiencies, but is not required and would only involve the root initiation step to enhance the number of transformed roots yielding chimeric plants and not to obtain and select for stably transformed whole plants. Thus, this rejection should be withdrawn.

The Examiner has also rejected claim 5 under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described adequately in the specification. Applicants' respectfully traverse and request reconsideration. The *Agrobacterium rhizogenes* strain is known and available in the art as identified in the enclosed reference of Savka et al (Phytopathology, Vol. 80, No. 5, 1990, pp. 504). This strain is just one of many strains that could be used with utility in the claimed invention. Claim 5 merely recites a particular strain. Thus, no deposit is required and this rejection should be withdrawn.

Rejection based on 35 U.S.C. § 102(b)

Claims 1-4, 6 and 8-11 stand rejected under 35 USC §102(b) as being anticipated by Trulson et al. Applicants respectfully traverse this rejection and request reconsideration in view of the following remarks.

Trulson does not disclose a method for producing a stably transformed chimeric plant having transformed root tissue and wild-type shoots, leaves, stems etc. In fact, Trulson is directed to obtaining a fully transformed *Cucumis* plant. As described on page 4, lines 53-65 and onto page 5, lines 1-7, the goal of Trulson was to obtain transgenic plants that were positive for nptII and producing germinable seed capable of being carried into further generations. There is no teaching of using Agrobacterium rhizogenes to obtain a chimeric plant with transformed root tissue and having the remaining plant tissues being wild-type.

Similarly, claims 1-4 and 6-7 stand rejected under 35 USC §102(b) as being anticipated byRech et al. Rech merely discloses a method for producing stable transformed plants expressing a selectable marker, but does not disclose production of stable transformed chimeric plants as discussed above. Thus, this rejection should also be withdrawn.

Rejection based on 35 U.S.C. § 103(a)

Claims 1-4 and 6-11 stand rejected under 35 USC 103(a) as being unpatentable over Rech et al in view of Hatamoto et al. Neither Rech nor Hatamoto teach, suggest or disclose a method for the production of a stably transformed chimeric plant having transgenic root tissue

and wild-type shoots, stems or leaves as specifically claimed by Applicants. Thus, the §103 rejection cannot stand.

In view of the foregoing, it is submitted that the newly amended claims are in condition for allowance. Reconsideration and withdrawal of the rejections is respectfully requested. If the examiner believes that a phone conference with Applicants' representative would advance the application to allowance, she is invited to telephone the undersigned at the number below.

Respectfully Submitted,

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29 May 02
Date 29 May 2002

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage paid, in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C., 20231 on 29 May 2002.

DATE: *29 May 2002*

Signature
Thomas P. McBride
Printed Name

IN THE CLAIMS:

1. (amended) A method for producing a [stable] stably transformed chimeric plant having transgenic root tissue, the method comprising the steps of:
obtaining an explant from a selected plant species;
[inoculating] transforming the explant with *Agrobacterium rhizogenes* containing an exogenous nucleic acid sequence [genetic element] capable of being transferred to the explant;
culturing the [inoculated] transformed explant in a root initiating media to produce [manner permitting transgenic] transformed roots [development]; and
transferring the transformed roots to soil or a hydroponic environment to produce the chimeric plant having transformed roots and wild type shoots, stems and leaves [producing a stable chimeric plant with transgenic root tissue].

3. (amended) The method of claim 1 wherein the explant is a hypocotyl [providing] having a cut end below the cotyledon.

8. (amended) The method of claim 4 wherein [transgenic] transformed roots [development is] are initiated in the [inoculated] hypocotyl by placing the [inoculated] end of the hypocotyl contacted with the *Agrobacterium rhizogenes* [region] in a media containing ¼ strength [MS] Murashige and Skoog media.

11. (amended) The method of claim 10 wherein the concentration of kanamycin in the media is no more than [about] 50 mg/L.

Webster's II

New College Dictionary

Houghton Mifflin Company
Boston • New York

) one's native land. —n
try. 2. One who has
ing in a foreign countr

ig, -pects. [Lat. exp
ecere. to see.] 1. To lo
arance of <expected
ted the rain to stop
ity from sales clerks
. To presume : supp
adv. —ex·pect'ed

es. 1. EXPECTATION
ased on statistical pro

or marked by expectati
NANT. —ex·pec'tan

The act of expecting
1. 3. a. expectation
The expected value of
ICY 2b.
elating to, or marked by

moting or facilitating
ct. —n. An expectoran

·ed, -rat·ing, -rates
the breast : ex-, out of
uth : SPIT. 2. To cough
clear the chest and lung
ec'to·ra'tion n.
ncy.
cies. 1. Appropriatenes
-serving means. 3. An

Lat. expediens, pr.part. of
1. Appropriate to a give
st. b. Based on or marke
e. 3. Obs. Speedy : exp
ntrivance for meeting

. Of or relating to what

·ing, -dites. [Lat. exp
x-, out + pes, foot.] 1. T
o perform efficiently and
·ex'pe·dit'er, ex'pe

HURRY, QUICKEN, STEP
of <expedite a delivery
legislature>
xpedicioun, military cam
< expedire, to extricat
n for a definite purpose
. Speed in performance

) adj. Relating to or con

cting or carried out with
·ly adv. —ex'pe·di'

, -pels. [ME expellen
1. To force or drive out.
e. 3. To dismiss, as from
—ex·pel'ler n.
el'ant) adj. Expelling

xpelled.
PELLANT.
ad·ing, -pends. [ME
ere, to pay.] 1. To lay out

subject to use or consum
n or maintenance. —ex

The act or process of
expense.
nse < Lat. expensa < fem
omething paid out to atta
ing given up for the sake
. a. Charges incurred by
duties. b. Informal. Mon

ē be hw which
for oi noise ōō took

allotted for payment of such charges. 3. Something requiring the expenditure of money. 4. *Archaic.* An expenditure. —*vt.* **-pensed, -pens·ing, -pens·es.** 1. To charge with expenses. 2. To write off as an expense.

expense account *n.* An account of expenses for repayment to an employee.

ex·pen·sive (ĭk-spĕn'sĭv) *adj.* Bringing a large price : COSTLY. **—ex·pen'sive·ly** *adv.* **—ex·pen'sive·ness** *n.*

★ ***syns:*** EXPENSIVE, COSTLY, DEAR, HIGH, PRICEY *adj. core meaning* : bringing a large price <*expensive* jewels> ***ant:*** CHEAP

ex·pe·ri·ence (ĭk-spîr'ē-əns) *n.* [ME < OFr. or < Lat. *experientia* < *experiens*, pr.part. of *experiri*, to try.] 1. Apprehension or perception of an object, thought, emotion, or event through the senses or mind. 2. a. Active participation in events or activities, leading to accumulation of knowledge or skill. b. The knowledge or skill so derived. 3. a. An event or series of events participated in. b. The totality of such events in the past of an individual or group. —*vt.* **-enced, -enc·ing, -enc·es.** To participate in personally : UNDERGO <*experienced* a sense of elation>

experience table *n.* A table compiled from life-insurance statistics to indicate longevity.

ex·pe·ri·en·tial (ĭk-spîr'ē-ĕn'shəl) *adj.* Relating to or derived from experience. **—ex·pe'ri·en'tial·ly** *adv.*

ex·per·i·ment (ĭk-spĕr'ə-mənt) *n.* [ME < OFr. or < Lat. *experimentum* < *experiri*, to try.] 1. A test performed to demonstrate a known truth, examine the validity of a hypothesis, or ascertain the efficacy of something previously untried. 2. The conducting of a test. —*vi.* (-mĕnt') **-ment·ed, -ment·ing, -ments.** To conduct an experiment. **—ex·per'i·ment'er** *n.*

ex·per·i·men·tal (ĭk-spĕr'ə-mĕn'tl) *adj.* 1. a. Of, relating to, or based on experiment. b. Given to experimenting. 2. Of the nature of an experiment. 3. Proven by experience : EMPIRICAL. **—ex·per'i·men'tal·ly** *adv.*

ex·per·i·men·tal·ism (ĭk-spĕr'ə-mĕn'tl-ĭz'əm) *n.* Use of experimental methods in determining the validity of an idea. **—ex·per'i·men'tal·ist** *n.*

ex·per·i·men·ta·tion (ĭk-spĕr'ə-mĕn-tā'shən) *n.* The act, process, or practice of experimenting.

experiment station *n.* An establishment in which scientific experiments are conducted in a specific field, as agriculture, and practical applications are developed.

ex·pert (ĕk'spûrt') *n.* [ME < OFr., experienced < Lat. *expertus*, p.part. of *experiri*, to try.] 1. A person with a high degree of skill in or knowledge of a specific subject. 2. a. The highest grade that can be achieved in marksmanship. b. One who has achieved this grade. —*adj.* (ĕk'spûrt, ĭk-spûrt'). Having or displaying great skill, dexterity, or knowledge as the result of experience. **—ex'pert'ly** *adv.* **—ex'pert'ness** *n.*

ex·per·tise (ĕk'spûr-tēz') *n.* [Fr. < OFr. < *expert*, experienced. —see EXPERT.] 1. Expert opinion or advice. 2. Specialized knowledge or skill : MASTERY.

ex·pi·a·ble (ĕk'spē-ə-bəl) *adj.* Capable of being expiated.

ex·pi·ate (ĕk'spē-āt') *v.* **-at·ed, -at·ing, -ates.** [Lat. *expiare, expiat-* : *ex-* (intensive) + *piare*, to atone < *pius*, devout.] —*vt.* To make atonement for. —*vi.* To make expiation. **—ex'pi·a'tor** *n.*

ex·pi·a·tion (ĕk'spē-ā'shən) *n.* 1. The act of expiating : ATONEMENT. 2. Means of atonement. **—ex'pi·a·to'ry** (-ə-tôr'ē, -tōr'ē) *adj.*

ex·pi·ra·tion (ĕk'spə-rā'shən) *n.* 1. The act of coming to a close : TERMINATION. 2. The act of breathing out. 3. *Obs.* Death.

ex·pi·ra·to·ry (ĭk-spī'rə-tôr'ē, -tōr'ē) *adj.* Of, relating to, or involving the expiration of air from the lungs.

ex·pire (ĭk-spīr') *v.* **-pired, -pir·ing, -pires.** [ME *expiren* < Lat. *exspirare* : *ex-*, out + *spirare*, to breathe.] —*vi.* 1. To come to an end : TERMINATE <My subscription has *expired.*> 2. To die. 3. To breathe out : EXHALE. —*vt.* 1. To breathe out from or as if from the lungs. 2. *Archaic.* To give off.

ex·pi·ry (ĭk-spīr'ē) *n., pl.* **-ries.** 1. An expiration, esp. of a contract or agreement. 2. Death.

ex·plain (ĭk-splān') *v.* **-plained, -plain·ing, -plains.** [ME *explanen* < Lat. *explanare* : *ex-* (intensive) + *planus*, clear.] —*vt.* 1. To make understandable. 2. To define : expound <We *explained* our plan.> 3. To offer reasons for or a cause of : JUSTIFY <*explain* an absence> —*vi.* To provide an explanation. **—explain away.** 1. To dismiss by or as if by explaining. 2. To minimize by explanation. **—ex·plain'a·ble** *adj.*

ex·pla·na·tion (ĕk'splə-nā'shən) *n.* 1. The act or process of explaining. 2. Something that explains. 3. Mutual clarification of misunderstandings : RECONCILIATION.

ex·plan·a·tive (ĭk-splăn'ə-tĭv) *adj.* Explanatory. **—ex·plan'a·tive·ly** *adv.*

ex·plan·a·to·ry (ĭk-splăn'ə-tôr'ē, -tōr'ē) *adj.* Serving or intended to explain. **—ex·plan'a·to'ri·ly** *adv.*

ex·plant (ĕk-splănt') *vt.* **-plant·ed, -plant·ing, -plants.** To take (living tissue) from the natural site of growth and place in a medium or culture. —*n.* Material explanted. **—ex'plan·ta'tion** *n.*

ex·ple·tive (ĕk'splĭ-tĭv) *n.* [< LLat. *expletivus*, serving to fill out < Lat. *expletus* < *explēre*, to fill out : *ex-*, out + *plēre*, to fill.] 1. An often profane or obscene exclamation. 2. a. An added word or phrase that does not contribute meaning but serves to fill out a sentence or metrical line. b. A word standing in place of and anticipating a following word or phrase; e.g., in the sentence "*There are many books on the table*," the word *there* is an expletive. —*adj.* Added to fill out something, as a metrical line or sentence.

ex·ple·to·ry (ĕk'splĭ-tôr'ē) *adj.* Expletive.

ex·pli·ca·ble (ĕk'splĭ-kə-bəl) *adj.* Capable of being explained. **—ex'pli·ca·bly** *adv.*

ex·pli·cate (ĕks'plĭ-kāt') *vt.* **-cat·ed, -cat·ing, -cates.** [Lat. *explicare*, to unfold : *ex-*, out + *plicare*, to fold.] To make clear the meaning of : EXPLAIN. **—ex·pli·ca'tion** *n.* **—ex'pli·ca'tor** *n.*

ex·pli·ca·tion de texte (ĕk-splē-kä-syôN' də tĕkst') *n., pl.* **ex·pli·ca·tions de texte** (ĕk-splē-kä-syôN' də tĕkst') [Fr. : *explication*, explanation + *de*, of + *texte*, text.] A method of literary criticism involving intense analysis and exhaustive interpretation of each part of the work.

ex·pli·ca·tive (ĕk'splĭ-kə-tĭv) *adj.* Explanatory. **—ex'pli·ca·tive** *n.* **—ex'pli·ca·tive·ly** *adv.*

ex·plic·it (ĭk-splĭs'ĭt) *adj.* [Fr. *explicite* < Lat. *explicitus*, p.part. of *explicare*, to unfold. —see EXPLICATE.] 1. a. Expressed without vagueness or ambiguity : SPECIFIC. b. Clearly formulated or defined. 2. Forthright and unreserved in expression. **—ex·plic'it·ly** *adv.* **—ex·plic'it·ness** *n.*

ex·plode (ĭk-splōd') *v.* **-plod·ed, -plod·ing, -plodes.** [Lat. *explodere*, to drive out by clapping : *ex-*, out + *plaudere*, to clap.] —*vi.* 1. To release mechanical, chemical, or nuclear energy in an explosion. 2. To burst violently from internal pressure. 3. To burst forth suddenly and often violently. 4. To increase suddenly, sharply, and without control. —*vt.* 1. To cause to explode or burst violently and noisily. 2. To show to be unreliable or false <*explode* a theory> 3. *Obs.* To drive off the stage by the unrestrained expression of dissatisfaction. **—ex·plod'er** *n.*

★ ***syns:*** EXPLODE, BLAST, BLOW UP, BURST, DETONATE, GO OFF *v. core meaning* : to release energy violently and suddenly, esp. with a loud report <a bomb that *exploded* in midair>

exploded view *n.* An illustration or diagram of a construction that shows its parts separately but in positions that indicate their proper relationships to the whole.

ex·ploit (ĕk'sploit', ĭk-sploit') *n.* [ME < OFr. < Lat. *explicitum*, neuter p.part. of *explicare*, to explicate.] An act or deed, esp. a brilliant or heroic feat. —*vt.* (ĭk-sploit', ĕk'sploit') **-ploit·ed, -ploit·ing, -ploits.** 1. To utilize to the greatest possible advantage. 2. To make use of unethically or selfishly <*exploiting* the employees> **—ex·ploit'a·ble** *adj.* **—ex·ploit'a·tive** *adj.* **—ex·ploit'er** *n.*

ex·ploi·ta·tion (ĕk'sploi-tā'shən) *n.* 1. An act of exploiting. 2. Utilization of another person for selfish purposes. 3. A publicity or advertising program.

ex·plore (ĭk-splôr', -splōr') *v.* **-plored, -plor·ing, -plores.** [Lat. *explorare.*] —*vt.* 1. To investigate systematically : EXAMINE <*explore* every suggestion given> 2. To search into or range over for the purpose of discovery. 3. *Med.* To examine for diagnostic purposes. —*vi.* To make a careful search or examination. **—ex'plo·ra'tion** (ĕk'splə-rā'shən) *n.* **—ex·plor'a·to'ry** (ĭk-splôr'ə-tôr'ē, -splōr'ə-tōr'ē) *adj.*

ex·plor·er (ĭk-splôr'ər, -splōr'-) *n.* 1. One who explores, esp. one who explores a geographic area. 2. An implement used for exploring : PROBE.

ex·plo·sion (ĭk-splō'zhən) *n.* [Lat. *explosio* < *explodere*, to drive out by clapping. —see EXPLODE.] 1. a. An act or instance of exploding. b. The loud, sharp sound made by an explosion. 2. A sudden, often vehement outburst, as of emotion. 3. A sudden and great increase <the population *explosion*> 4. Plosion.

ex·plo·sive (ĭk-splō'sĭv) *adj.* [< Lat. *explodere, explos-*, to drive out by clapping. —see EXPLODE.] 1. Relating to or of the nature of an explosion. 2. Tending to explode. —*n.* 1. A substance, esp. a prepared chemical, that explodes or causes explosion. 2. STOP 12. **—ex·plo'sive·ly** *adv.* **—ex·plo'sive·ness** *n.*

ex·po·nent (ĭk-spō'nənt, ĕk'spō'nənt) *n.* [Lat. *exponens, exponent-*, pr.part. of *exponere*, to put forward : *ex-*, out + *ponere*, to put.] 1. One that expounds or interprets. 2. One that speaks for, represents, or advocates. 3. *Math.* A number or symbol, as 3 in $(x+y)^3$, placed to the right of and above another number, symbol, or expression and denoting the power to which the latter is to be raised. —*adj.* Expository : explanatory.

ex·po·nen·tial (ĕk'spə-nĕn'shəl) *adj.* 1. *Math.* a. Containing, involving, or expressed as an exponent. b. Expressed in terms of a designated power of *e*, the base of natural logarithms. 2. Of or relating to an exponent. **—ex'po·nen'tial·ly** *adv.*

ex·po·nen·ti·a·tion (ĕk'spə-nĕn'shē-ā'shən) *n. Math.* The act of raising a quantity to a power.

ex·port (ĭk-spôrt', -spōrt', ĕk'spôrt', -spōrt') *v.* **-port·ed, -port·ing, -ports.** [Lat. *exportare* : *ex-*, out + *portare*, to carry.] —*vt.* To send or transport (e.g., a commodity) abroad, esp. for sale or trade.

en, a sign indicating a com-
), one.] A punctuation mark
id word or between syllables
l of a line. —vt. -phened,
at·ing, -ates. To connect
th a hyphen. —hy'phen-
hĭp'nə-gŏj'ĭk, -gō'jĭk) adj.
Gk. agōgos, leading < agein
ng to the period or state of
s. sleep.] 1. Sleep <hypno
s) n. Psychoanalytic treat
The process of inducing a
-net'ic (-jə-nĕt'ĭk) adj.
adj. var. of HYPNAGOGIC
al (hĭp-noid'l) adj. Of or
Abnormal fear of sleep
. [HYPNO- + Gk. pompé, s
artially conscious period o
The god of sleep.
ēz'). 1. An artificially in
ividual is receptive to sug
rpnotism. 3. A sleeplike
. Therapy based on or using
que < LLat. hypnoticus
) < hupnos, sleep.] 1. a. Of
hypnotism. 2. Inducing or
a. One who is hypnotized
: causing sleep : SOPORIFIC
heory or practice of induc-
sis. —hyp'no-tist n.
iz·ing, -iz·es. 1. To put
or as if by hypnosis <hyp
eld wipers> —hyp'no-
. —hyp'no·tiz'er n.
Sodium thiosulfate.
hypodermic syringe. 2. A
under, beneath.] 1. Below
ial : deficient <hypoesth
ypoxanthine>
. Chem. Slight acidity. 2
normal pressure. —hy'
last. —hy'po·blas'tic
caustum < Gk. hupokau
hupo, beneath + kaiein, to
from a furnace was accu
Rome.
rface position directly be
hy'po·cen'tral adj.
n. A salt or ester of hy
klôr'-) n. A weak, unsta
and used as a deodorant
[LLat., abdomen (the seat
hupokhondrion, abdomen
: breastbone : hupo, under
urotic conviction that one
experiences of actual pain
. pl. of HYPOCHONDRIUM
n. One afflicted with hy
g hypochondria. 2. Anat
. —hy'po·chon·dri'-
a·dri'a·cal·ly adv.
-sĭs) n. [HYPOCHONDRIA
a) n., pl. -dr·ia (-drē-ə)

ē be hw which ĭ pit
or oi noise ōō took

...[NLat. < Gk. hupokhondrion, abdomen. —see HYPOCHONDRIA.] The upper lateral region of the abdomen, below the lowest ribs.

hy·poc·o·rism (hī-pŏk'ə-rĭz'əm, hī'pə-kôr'ĭz'əm, -kōr'-) n. [LLat. hypocorisma < Gk. hupokorisma < hupokorizesthai, to call by endearing names : hypo, below + korizesthai, to caress < koros, boy, and korē, girl.] 1. A name of endearment : pet name. 2. The use of pet names. —**hy'po·co·ris'tic** (hī'pə-kə-rĭs'tĭk), **hy'po·co·ris'ti·cal** adj. —**hy'po·co·ris'ti·cal·ly** adv.

hy·po·cot·yl (hī'pə-kŏt'l) n. [HYPO- + COTYL(EDON).] The part of the axis of a plant embryo or seedling plant below the cotyledons.

hy·poc·ri·sy (hĭ-pŏk'rĭ-sē) n., pl. **-sies.** [ME ipocrisie < OFr. < LLat. hypocrisis < Gk. hupokrisis, pretense < hupokrinesthai, to pretend : hupo-, from under + krinesthai, to explain.] 1. The practice of professing feelings, beliefs, or virtues one does not hold or possess : INSINCERITY. 2. An act or instance of hypocrisy.

hyp·o·crite (hĭp'ə-krĭt') n. [ME ipocrite < OFr. < LLat. hypocrita < Gk. hupocritēs, actor < hupokrinein, to play a part. —see HYPOCRISY.] One given to hypocrisy or dissemblance.

hyp·o·crit·i·cal (hĭp'ə-krĭt'ĭ-kəl) adj. 1. Marked by hypocrisy. 2. Being a hypocrite. —**hyp'o·crit'i·cal·ly** adv.

hy·po·cy·cloid (hī'pō-sī'kloid') n. The plane locus of a point fixed on a circle that rolls on the inside circumference of a fixed circle.

hy·po·derm (hī'pə-dûrm') n. var. of HYPODERMIS.

hy·po·der·mal (hī'pə-dûr'məl) adj. 1. Of or relating to the hypodermis. 2. Lying beneath the epidermis.

hy·po·der·mic (hī'pə-dûr'mĭk) adj. [HYPO- + DERM(ATO)- + -IC.] 1. Of or relating to the layer just beneath the epidermis. 2. Relating to the hypodermis. 3. Injected beneath the skin. —n. 1. A hypodermic injection. 2. A hypodermic needle. 3. A hypodermic syringe. —**hy'po·der'mi·cal·ly** adv.

hypodermic injection n. A subcutaneous, intramuscular, or intravenous injection by means of a hypodermic syringe and needle.

hypodermic needle n. 1. A hollow needle used with a hypodermic syringe. 2. A hypodermic syringe.

hypodermic syringe n. A syringe fitted with a hypodermic needle for hypodermic injections.

hy·po·der·mis (hī'pə-dûr'mĭs) also **hy·po·derm** (hī'pə-dûrm') n. 1. Zool. An epidermal layer of cells that secretes an overlying chitinous cuticle, as in arthropods. 2. Bot. A layer of cells lying immediately beneath the epidermis.

hy·po·es·the·sia (hī'pō-ĭs-thē'zhə) also **hy·pes·the·sia** (hī'-...) n. [HYPO- + (AN)ESTHESIA.] Pathol. Partial loss of sensation.

hy·po·eu·tec·tic (hī'pō-yōō-tĕk'tĭk) adj. Chem. Having the minor component present in a smaller amount than in the eutectic composition of the same components.

hy·po·gas·tri·um (hī'pə-găs'trē-əm) n., pl. **-tri·a** (-trē-ə) [NLat. < Gk. hypogastrion : hupo, below + gastēr, belly.] The lowest of the three median regions of the abdomen. —**hy'po·gas'tric** adj.

hy·po·ge·a (hī'pə-jē'ə) n. pl. of HYPOGEUM.

hy·po·ge·al (hī'pə-jē'əl) also **hy·po·ge·an** (-ən) or **hy·po·ge·ous** (-əs) adj. [< LLat. hypogeus < Gk. hupogaios : hupo, below + gaia, earth.] 1. Located beneath the earth's surface : UNDERGROUND. 2. Bot. Designating or marked by cotyledons remaining below the surface of the ground. —**hy'po·ge'al·ly** adv.

hy·po·gene (hī'pə-jēn') adj. [HYPO- + (EPI)GENE.] Formed or situated below the earth's surface <hypogene rocks>

hy·pog·e·nous (hī-pŏj'ə-nəs) adj. Bot. Developing or growing on a lower surface, as fungi on leaves.

hy·po·ge·ous (hī'pə-jē'əs) adj. var. of HYPOGEAL.

hy·po·ge·um (hī'pə-jē'əm) n., pl. **-ge·a** (-jē'ə) [Lat. < Gk. hupogaion < hupogaios, hypogeal.] 1. A subterranean chamber of an ancient building. 2. An ancient subterranean burial chamber, as a catacomb.

hy·po·glos·sal (hī'pə-glŏs'əl) adj. [< NLat. hypoglossus, hypoglossal nerve : HYPO- + Gk. glōssa, tongue.] Anat. Of or relating to the hypoglossal nerve. —n. The hypoglossal nerve.

hypoglossal nerve n. A motor nerve attached to the medulla oblongata and innervating the muscles of the tongue.

hy·po·gly·ce·mi·a (hī'pō-glī-sē'mē-ə) n. An abnormally low level of glucose in the blood. —**hy'po·gly·ce'mic** adj.

hy·pog·y·nous (hī-pŏj'ə-nəs) adj. Bot. Having or characterizing floral organs or parts that are below and not in contact with the ovary. —**hy·pog'y·ny** (-nē) n.

hy·po·ma·ni·a (hī'pə-mā'nē-ə, -mān'yə) n. A mild state of mania involving slightly abnormal overactivity and elation. —**hy'po·man'ic** (-măn'ĭk) adj.

hy·po·nas·ty (hī'pə-năs'tē) n. An upward bending of plant parts, as leaves, caused by growth of the lower side. —**hy'po·nas'tic** adj.

hy·po·phos·phite (hī'pō-fŏs'fīt') n. A salt of hypophosphorous acid.

hy·po·phos·pho·rous acid (hī'pō-fŏs'fər-əs, -fŏs-fôr'əs, -fōr'-) n. A clear, colorless or slightly yellow liquid, H_3PO_2, used in preparing hypophosphites.

hy·poph·y·sis (hī-pŏf'ĭ-sĭs) n., pl. **-ses** (-sēz') [NLat. < Gk. hupophusis, attachment underneath < hupophein, to grow beneath : hupo, beneath + phuein, to grow.] The pituitary gland. —**hy·poph'y·se'al** (-sē'əl), **hy'po·phys'i·al** (hī'pə-fĭz'ē-əl) adj.

hy·po·pi·tu·i·ta·rism (hī'pō-pĭ-tōō'ĭ-tə-rĭz'əm, -tyōō'-) n. Deficient or decreased production of pituitary hormones. —**hy'po·pi·tu'i·tar'y** (-tĕr'ē) adj.

hy·po·pla·sia (hī'pō-plā'zhə) n. Pathol. Incomplete or arrested development of an organ or part. —**hy'po·plas'tic** (-plăs'tĭk) adj.

hy·po·ploid (hī'pō-ploid') adj. Genetics. Having a chromosome number less by only a few chromosomes than the normal diploid number. —**hy'po·ploi'dy** n.

hy·po·pne·a (hī'pō-nē'ə) n. [HYPO- + Gk. pnoē, breath < pnein, to breathe.] Abnormally slow and shallow breathing.

hy·po·sen·si·tiv·i·ty (hī'pō-sĕn'sĭ-tĭv'ĭ-tē) n. Less than normal sensitivity. —**hy'po·sen'si·tive** adj.

hy·po·sen·si·tize (hī'pō-sĕn'sĭ-tīz') vt. **-tized, -tiz·ing, -tiz·es.** To make less sensitive. —**hy'po·sen'si·ti·za'tion** n.

hy·pos·ta·sis (hī-pŏs'tə-sĭs) n., pl. **-ses** (-sēz') [LLat. < Gk. hupostasis : hupo, beneath + stasis, a standing.] 1. Philos. The substance or essence of something. 2. a. Any of the persons of the Trinity. b. The essential person of Christ in which His human and divine natures are united. 3. An entity that has been hypostatized. 4. a. A settling of solid particles in a fluid. b. Something that settles to the bottom of a fluid : SEDIMENT. 5. A condition in which the action of one gene conceals or suppresses the action of another gene that is not its allele but that affects the same organ, part, or state of the body. —**hy'po·stat'ic** (hī'pə-stăt'ĭk), **hy'po·stat'i·cal** adj. —**hy'po·stat'i·cal·ly** adv.

hy·pos·ta·tize (hī-pŏs'tə-tīz') vt. **-tized, -tiz·ing, -tiz·es.** [< Gk. hupostatos, standing under < hyphistasthai, to stand under : hupo, beneath + histasthai, to stand.] 1. To symbolize (a concept) in a material form. 2. To ascribe material existence to. —**hy·pos'ta·ti·za'tion** n.

hy·po·sthe·ni·a (hī'pəs-thē'nē-ə) n. [HYPO- + Greek sthenos, strength.] Abnormal weakness. —**hy'po·sthen'ic** (-thĕn'ĭk) adj.

hy·po·style (hī'pə-stīl') n. [< Gk. hupostulos, resting upon pillars : hupo, beneath + stulos, pillar.] A building having a roof or ceiling supported by rows of columns. —**hy'po·style'** adj.

hy·po·sul·fite (hī'pō-sŭl'fīt') n. Sodium thiosulfate.

hy·po·sul·fu·rous acid (hī'pō-sŭl-fyŏŏr'əs, -sŭl'fər-əs) n. An unstable acid, $H_2S_2O_4$, known only in aqueous solution and used as a bleaching and reducing agent.

hy·po·tax·is (hī'pə-tăk'sĭs) n. [Gk. hupotaxis, subjection < hupotassein, to arrange under : hupo, under + tattein, to arrange.] The dependent or subordinate relationship of clauses with connectives. —**hy'po·tac'tic** (-tăk'tĭk) adj.

hy·po·ten·sion (hī'pō-tĕn'shən) n. Abnormally low blood pressure.

hy·pot·e·nuse (hī-pŏt'n-ōōs', -yōōs') also **hy·poth·e·nuse** (-pŏth'ə-nōōs', -nyōōs') n. [Lat. hypotenusa < Gk. hupoteinousa < hupoteinein, to stretch under : hupo, under + teinein, to stretch.] The side of a right triangle opposite the right angle.

hy·po·thal·a·mus (hī'pō-thăl'ə-məs) n. The part of the brain that lies below the thalamus, forming the major portion of the ventral region of the diencephalon and functioning to regulate autonomic activities, as bodily temperature and certain metabolic processes. —**hy'po·tha·lam'ic** (-thə-lăm'ĭk) adj.

hy·poth·e·cate (hī-pŏth'ĭ-kāt') vt. **-cat·ed, -cat·ing, -cates.** [Med. Lat. hypothecare, hypothecat- < LLat. hypotheca, pledge < Gk. hupothēkē < hupotithenai, to give as a pledge : hupo, beneath + tithenai, to place.] To pledge (property) as security or collateral for a debt without transfer of title or possession. —**hy·poth'e·ca'tion** n. —**hy·poth'e·ca'tor** (-kā'tər) n.

hy·poth·e·nuse (hī-pŏth'ə-nōōs', -nyōōs') n. var. of HYPOTENUSE.

hy·po·ther·mal (hī'pō-thûr'məl) adj. Geol. Of, relating to, or being high-temperature deposits derived from magmatic emanations forced under pressure into place in pre-existing rock openings.

hy·po·ther·mi·a (hī'pō-thûr'mē-ə) n. [NLat. : HYPO- + Gk. thermē, heat.] Abnormally low body temperature. —**hy'po·ther'mic** adj.

hy·poth·e·sis (hī-pŏth'ĭ-sĭs) n., pl. **-ses** (-sēz') [Gk. hupothesis < hupotithenai, to suppose : hupo, beneath + tithenai, to place.] 1. An explanation accounting for a set of facts that can be tested by further investigation : THEORY. 2. Something considered to be true for the purpose of investigation or argument : ASSUMPTION.

hy·poth·e·size (hī-pŏth'ĭ-sīz') vt. & vi. **-sized, -siz·ing, -siz·es.** To assert as or form a hypothesis.

hy·po·thet·i·cal (hī'pə-thĕt'ĭ-kəl) also **hy·po·thet·ic** (-thĕt'ĭk) adj. [Gk. hupothetikos < hupothesis, hypothesis.] 1. Of, relating to, or based on a hypothesis. 2. a. Conjectural : suppositional. b. Contingent : conditional. —**hy'po·thet'i·cal·ly** adv.

hy·po·thy·roid (hī'pō-thī'roid') adj. Affected by or exhibiting hypothyroidism.

hy·po·thy·roid·ism (hī'pō-thī'roi-dĭz'əm) n. 1. Insufficient production of thyroid hormones. 2. A pathological condition, esp. cretinism or myxedema, resulting from severe thyroid insufficiency.

hy·po·ton·ic (hī'pō-tŏn'ĭk) adj. 1. Pathol. Having less than nor-

move in a set course. 3. To be capable of being steered or guided <a car that *steers* easily> —*n.* A piece of advice. **—steer′a·ble** *adj.* **—steer′er** *n.*

steer² (stîr) *n.* [ME < OE *stēor.*] A young ox castrated before sexual maturity and raised for beef.

steer·age (stîr′ĭj) *n.* 1. The act or practice of steering. 2. A ship's steering mechanism. 3. The section of a passenger ship, orig. near the rudder, providing the cheapest passenger accommodations.

steer·age·way (stîr′ĭj-wā′) *n.* The minimum rate of motion required for the helm of a ship or boat to have effect.

steering committee *n.* A committee that sets agendas and schedules business, as for a legislative body.

steering gear *n.* The mechanism by which dispositions of the steering controls of a vehicle are transferred to the part that interacts with the external medium.

steering wheel *n.* A wheel that controls steering.

steers·man (stîrz′mən) *n.* A helmsman.

steeve¹ (stēv) *n.* [ME *steven,* to stow < OFr. *estiver* < Sp. *estibar,* to cram < Lat. *stipare.*] A derrick or spar with a block at one end, used for stowing cargo. —*vt.* **steeved, steev·ing, steeves.** To pack or stow (cargo) in the hold of a ship.

steeve² (stēv) [Orig. unknown.] —*n. Naut.* The angle formed by the bowsprit and the horizon or the keel. —*v.* **steeved, steev·ing, steeves.** —*vt.* To incline (a bowsprit) upward at an angle with the horizon or the keel. —*vi.* To have an upward inclination. —Used of a bowsprit.

steg·o·don (stĕg′ə-dŏn′) *also* **steg·o·dont** (-dŏnt′) *n.* [NLat. *Stegodon,* genus name : Gk. *stegos,* roof (< *stegein,* to cover) + Gk. *odous, odont-,* tooth.] An extinct elephantlike mammal of the genus *Stegodon* and of related genera, of the Pliocene to Pleistocene epochs.

steg·o·saur (stĕg′ə-sôr′) *also* **steg·o·sau·rus** (stĕg′ə-sôr′əs) *n.* [NLat. *Stegosaurus,* genus name : Gk. *stegos,* roof (< *stegein,* to cover) + *osaurs,* lizard.] An herbivorous dinosaur of the genus *Stegosaurus* and of related genera, of the Triassic to the Cretaceous periods, that had a double row of upright bony plates along the back.

stein (stīn) *n.* [G., prob. short for *Steingut,* stoneware : *Stein,* stone + *Gut,* goods.] A usu. one-pint mug, esp. for beer.

stein·bok (stīn′bŏk′) *n. var. of* STEENBOK.

ste·le (stē′lē) *n., pl.* **-les** *or* **-lae** (-lē) [Gk. *stēlē,* pillar.] 1. An upright stone or slab with an inscribed or sculptured surface, used as a monument or as a commemorative tablet in the face of a building. 2. *Bot.* The central core of vascular tissue in a plant stem. **—ste′lar** (-lər) *adj.*

stel·lar (stĕl′ər) *adj.* [Lat. *stella,* star.] 1. Of, relating to, or consisting of stars. 2. a. Of or relating to a star performer. b. Outstanding <a *stellar* performance>

stellar wind *n.* The varying flow of plasma ejected from a star's surface into interstellar space.

stel·late (stĕl′āt′) *also* **stel·lat·ed** (-ā′tĭd) *adj.* [Lat. *stellatus* < *stella,* star.] Arranged or shaped like a star <a *stellate* leaf> **—stel′late·ly** *adv.*

stel·li·form (stĕl′ə-fôrm′) *adj.* [NLat. *stelliformis* < Lat. *stella,* star.] Stellate.

stel·li·fy (stĕl′ə-fī′) *vt.* **-fied, -fy·ing, -fies.** [ME *stellifien* < OFr. *stellifier* < Med. Lat. *stellificare* : Lat. *stella,* star + Lat. *facere,* to make.] To transform into a star.

stel·lu·lar (stĕl′yə-lər) *adj.* [< LLat. *stellula,* dim. of Lat. *stella,* star.] 1. Having the form of a small star. 2. Bespangled with small stars.

St. El·mo's fire (sānt′ ĕl′mōz) *n.* Saint Elmo's fire.

stem¹ (stĕm) *n.* [ME < OE *stefn,* prow.] 1. a. The main ascending axis of a plant : a stalk or trunk. b. A slender stalk supporting or connecting another plant part, as a leaf or flower. 2. A banana stalk yielding several bunches of bananas. 3. A connecting or supporting part, esp.: a. The tube of a tobacco pipe. b. The slender upright support of a wine goblet. c. The small projecting shaft with an expanded crown by which a watch is wound. d. The rounded rod in the center of a lock about which a key fits and is turned. e. The shaft of a feather or hair. f. The upright stroke of a typeface or letter. g. The vertical line extending from the head of a musical note. 4. The main line of genealogical descent. 5. The main part of a word to which affixes are added. 6. The curved upright beam at the fore of a vessel into which the hull timbers are scarfed to form the prow. 7. The tubular glass structure mounting the filament or electrodes in an incandescent bulb or vacuum tube. —*v.* **stemmed, stem·ming, stems.** —*vt.* 1. To remove the stem of. 2. To provide with a stem. 3. To make headway against. —*vi.* To derive from or originate in. **—from stem to stern.** From one end to another. **—stem′less** *adj.*

stem² (stĕm) *v.* **stemmed, stem·ming, stems.** [ME *stemmen* < ON *stemma.*] —*vt.* 1. To stop or hold back by or as if by damming : STANCH. 2. To plug or tamp (e.g., a blast hole). 3. To point (skis) inward. —*vi.* To point skis inward in order to slow down or turn.

stem cell *n.* An unspecialized cell that gives rise to a specific specialized cell, as a blood cell.

stem·ma (stĕm′ə) *n., pl.* **stem·ma·ta** (stĕm′ə-tə) *or* **stem·mas.** [Lat., garland < Gk. < *stephein,* to encircle.] 1. An ancient Roman scroll recording the genealogy of a family : FAMILY TREE. 2. A diagram showing the relationships of the manuscripts of a literary work.

stemmed (stĕmd) *adj.* 1. Having the stems removed. 2. P… with a stem <long-*stemmed* roses>

stem·mer (stĕm′ər) *n.* One that removes stems, as from frui… bacco.

stem rust *n.* A rust disease affecting the stem of a plant.

stem·son (stĕm′sən) *n.* [STEM (prow) + (KEEL)SON.] *Naut.* A … supporting timber bolted to the stem and keelson at their … near the bow of a wooden vessel.

stem turn *n.* A skiing turn made by stemming the uphill … increasing one's weight upon it while bringing the other ski into … allel position.

stem·ware (stĕm′wâr′) *n.* Glassware mounted on a stem, …

stem-wind·er (stĕm′wīn′dər) *n.* A stem-winding watch.

stem-wind·ing (stĕm′wīn′dĭng) *adj.* Wound by turning … panded crown on the stem.

stench (stĕnch) *n.* [ME < OE *stenc,* odor.] A strong foul odor.

sten·cil (stĕn′səl) *n.* [< ME *stanselen,* to adorn with bright … OFr. *estenceler* < *estencele,* spark < VLat. **stincilla,* alteration … *scintilla,* spark.] 1. A sheet of plastic, cardboard, or other mat… which a desired lettering or design has been cut so that ink or … applied to the sheet will reproduce the pattern on the surface be… 2. The lettering or design produced by stencil. —*vt.* **-ciled, … ing, -cils** *or* **-cilled, -cil·ling, -cils.** 1. To mark with a ste… To make by stencil. **—sten′cil·er** *n.*

stencil paper *n.* Strong tissue-thin paper for making stencil…

sten·o (stĕn′ō) *n., pl.* **-os.** 1. A stenographer. 2. Stenograph…

steno– *pref.* [< Gk. *stenos,* narrow.] Narrow : small <*steno*…

sten·o·bath·ic (stĕn′ə-băth′ĭk) *adj.* Of or relating to an or… able to live only within a narrow range of water depths. **—ste… bath′** *n.*

sten·o·graph (stĕn′ə-grăf′) *n.* [Back-formation < STENOGRA…] A keyboard machine for reproducing letters in a shorthand syst… A character in shorthand. —*vt.* **-graphed, -graph·ing, -gr…** To write in shorthand.

ste·nog·ra·pher (stə-nŏg′rə-fər) *n.* One skilled in shorthand… one hired to take and transcribe dictation.

ste·nog·ra·phy (stə-nŏg′rə-fē) *n.* 1. The art or process of wri… shorthand. 2. Material in shorthand. **—sten′o·graph′ic** (… grăf′ĭk), **sten′o·graph′i·cal** *adj.* **—sten′o·graph′i…** *adv.*

sten·o·ha·line (stĕn′ə-hā′līn, -hăl′ĭn) *adj.* Of or relating to … ganism able to live only within a narrow range of water salinity.

ste·noph·a·gous (stə-nŏf′ə-gəs) *adj.* Feeding on a single … limited range of food.

ste·nosed (stə-nōzd′, -nōst′) *adj.* [STENOS(IS) + -ED.] Marked by … nosis.

ste·no·sis (stə-nō′sĭs) *n.* [NLat. < Gk. *stenōsis,* a narrowing … *noun,* to narrow < *stenos,* narrow.] Constriction of a passage or … **—ste·not′ic** (-nŏt′ĭk) *adj.*

sten·o·ther·mal (stĕn′ə-thûr′məl) *adj.* Of or relating to … isms adapted to living only within a limited range of tempera…

sten·o·top·ic (stĕn′ə-tŏp′ĭk) *adj.* [STENO- + Gk. *topos,* … Having narrow limits of adaptation to environmental conditions.

sten·o·type (stĕn′ə-tīp′) *n.* [(STENO)GRAPHY + TYPE.] 1. A … or combination of symbols representing a sound, word, or phras… in shorthand. 2. A keyboard machine used to record dictation … phonetic system.

sten·tor (stĕn′tôr′) *n.* [NLat. *Stentor,* genus name, after *St*… Greek herald. —see STENTORIAN.] Any of several trumpet-… aquatic microorganisms of the genus *Stentor,* with cilia arou… oral cavity.

sten·to·ri·an (stĕn-tôr′ē-ən, -tōr′-) *adj.* [After *Stentor,* a … voiced Greek herald in the *Iliad,* a Homeric poem.] Very loud … ator who spoke with a *stentorian* voice>

step (stĕp) *n.* [ME < OE *stæp.*] 1. a. The single complete mov… of raising one foot and putting it down in another spot, as in w… b. Manner of walking : GAIT. c. A fixed pace or rhythm, as in … ing. d. The sound of a footstep. e. A footprint. 2. a. The distance … versed by moving one foot ahead of the other. b. A very short d… <Our house is just a *step* away.> c. **steps.** Course : path <fo… in their parents' *steps*> 3. a. A rest for the foot in ascending or … scending. b. **steps.** Stairs. 4. a. One of a series of actions or mea… undertaken to reach a goal. b. A stage in a process. 5. A degree in … ress or a grade or rank in a scale <a *step* ahead of our competitors… *Mus.* The interval that separates two successive tones of a sca… *Computer Sci.* A single instructor in a computer sequence. 8. … The block in which the heel of a mast is fixed. —*v.* **stepped, … ping, steps.** —*vi.* 1. To put or press the foot <*step* on the acc… ator> 2. To move or shift slightly by taking a step or two … forward> 3. To walk a short distance to a specified place or in a … ified direction <*step* over to the counter> 4. To move with the … a particular way <Let's *step* lively!> 5. To move into a new situ… by or as if by taking a single step <*stepped* into a life of hardship… To treat with arrogant indifference <always *stepping* on p…

10. Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.
11. O'Neill, N. R., Rush, M. C., Horn, N. L., and Carver, R. B. 1977. Aerial blight of soybean caused by *Rhizoctonia solani*. Plant Dis. Rep. 61:713-717.
12. SAS Institute. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc. Cary, NC. 956 pp.
13. Schneider, R. 1953. Untersuchungen uber Feuchtigkeitsanspruche parasitischer Pilze. Phytopathol. Z. 21:63-78.
14. Sinclair, J. B., and Backman, P. A. 1989. Compendium of Soybean Diseases. 3rd ed. The American Phytopathological Socity, St. Paul, MN. 106 pp.
15. Singh, R., Shukla, T. N., Dwivedi, R. P., Shukla, H. P., and Singh, P. N. 1974. Study n [illegible]bean blight caused by *Rhizoctonia solani*. Ind. J. Mycol. Plant Pathol. 4:101-103.
16. Singh, R. S., and Singh, B. 1955. Ro t r t and wilt of *Cyamopsis psoralioides* in relation to thick and thin sowing of the crop. Agra Univ. India J. Res. Sci. 4:379-385.
17. Wu, Lung-chi, and Lin, Yi-shan 1966. Rhizoctonia aerial blight of soybean caused by *Thanatephorus cucumeris*. Mem. Coll. Agric. National Taiwan University 9:57-69.
18. Yang, X. B. 1989. Ecology and epidemiology of Rhizoctonia foliar blights of soybean. Ph.D. thesis. Louisiana State University, Baton Rouge. 157 pp.
19. Yang, X. B., Snow, J. P., and Berggren, G. T. 1990. Analysis f epidemics of Rhizoctonia aerial blight of soybean in Louisiana. Phytopathology 80:386-392.

Techniques

Induction of Hairy Roots on Cultivated Soybean Genotypes and Their Use to Propagate the Soybean Cyst Nematode

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ABSTRACT

Savka, M. A., Ravillion, B., Noel, G. R., and Farrand, S. K. 1990. Induction of hairy roots on cultivated soybean genotypes and their use to propagate the soybean cyst nematode. Phytopathology 80:503-508.

Ten soybean (*Glycine max*) genotypes were evaluated for hairy root induction by four strains of *Agrobacterium rhizogenes*. Influence of inoculati n site was assessed by infecting hypocotyls and cotyledons on germinated seedlings. The presence of opines in extracts of cultured roots was used to score transformed roots. A cucumopine strain, K599, induced hairy roots on 37% of the cotyledons infected on the 10 genotypes tested. Transformed root development after infection of cotyledons with the mannopine strain 8196 occurred at a frequency of 3% on four genotypes. Agropine strains 1855 and A4 induced hairy roots on 1% of cotyledons of different genotypes. No opine-positive transformed roots were induced from hypocotyl inoculations with any *A. rhizogenes* strain-soybean genotype combination tested. However, adventitious roots containing no detectable opines developed from hypocotyl inoculations both at the wound site and at a region directly below the cotyledons. Transf rmed roots differentiated from globular callus at the wound site on cotyled ns infected with virulent *A. rhizogenes*. Opine-containing hairy roots were established permanently in tissue culture and exhibited typical hairy ro t morphologies and growth parameters. Infection of soybean cultivar Williams 82 hairy root cultures with second-stage juveniles or cysts f the soybean cyst nematode, *Heterodera glycines* race 3, led t the appearance of mature cysts about 3 wk later. The nematode was propagated by excising an infected root and transferring it to a fresh root culture.

Agrobacterium rhizogenes, the causal agent of hairy root disease, induces the proliferation of neoplastic, transformed roots (1,35,37). During infection, the T-region, a segment of the root-inducing (Ri) plasmid in *A. rhizogenes*, is transferred and stably integrated into the plant genome (5). Upon expression of this integrated T-DNA, transformed roots rapidly proliferate and synthesize certain low molecular weight carbon compounds called opines (25). F ur pine-type Ri plasmids have been identified. Agropine-, mannopine-, cucum pine- and mikimopine-type Ri plasmids harbored in strains of *A. rhizogenes* induce transformed roots which synthesize the strain-specific opines (7,11,13,26).

Recently, hairy r ot cultures have been used to cultivate obligate root parasites. *Plasmodiophora bras...e* Woronin and *Polymyxa betae* Keskin, both bligate r ot-inhabiting fungi, can be propagated on transformed root cultures f sugar beet (19). Infections with vesicular-arbuscular mycorrhizal fungi, *Glomus mosseae* Gerdemann & Trappe and *Gigaspora margarita* Beker & Hall, have been obtained on hairy root cultures of *Convolvulus sepium* L. (20). In addition, the root-knot nematode, *Meloidogyne javanica*, has been propagated on transformed root cultures f potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) (32). Such cultures are being used f r routine maintenance of the nematode and to study the parasitism f *M. javanica* by *Pas..uria penetrans* (Thorne) Sayre & Starr (33).

Soybean (*Glycine max* (L.) Merr.) is grown widely in the United States as a source of oil and high-protein meal. Annually, the soybean crop is valued at an estimated 11 billion dollars. *Heterodera glycines* Ichinohe, the s ybean cyst nematode, occurs in Canada, the Peoples' Republic of Chin . Colombia, Ind nesia, Japan, Korea, the Soviet Union, and throughout the s ybean producti n areas of the United States (29). This obligate ro t parasite is a maj r yield-limiting pest of soybean in the United States (12).

The soybean cyst nematode can be pagated gnot biotically on normal soybean root explants (14). However, this technique requires the c ntinual establishment of r t explants because these rgans have a determinant period f gr wth in culture. Soybean hairy roots, which sh uld exhibit indeterminate growth in tissue culture, could provide an alternative t normal root explants for monoxenic pr pagati n and study of obligate soybean root parasites such as the soybean cyst nematode.

The few reports in the literature suggest that *A. rhizogenes*-induced hairy roots are difficult to establish n soybean. Responses of 26 genotypes of *G. max* to induction of hairy roots by *Agrobacterium* strain A136 harboring pRiA4b have been reported (23). Seven of the genotypes produced roots at the infecti n sites, another eight produced only small galls, and the remaining 11 did not respond to inoculations with this bacterial strain. However, attempts to culture these roots were unsuccessful. In addition, primary roots were not characterized with respect to pine content or other hairy root markers (23). Recently, Rech and co-workers (28) induced hairy roots on *G. canescens*, a wild *Glycine* spp. Permanent cultures could be established and the transformed roots were regenerable. However, hairy root cultures f the dc nesticated genotypes of *G. max* have not yet been reported.

This paper describes 1) an investigation into genotype, pathogen, and infection parameters necessary to induce hairy roots on *G. max*, 2) the establishment and characteristics of soybean hairy root cultures, and 3) the use of these cultures for the axenic propagation of the soybean cyst nematode.

MATERIALS AND METHODS

Soybean genotypes. The 10 genotypes of *Glycine max* used in this study were acquired from R. L. Bernard, curator, USDA Northern Soybean Germplasm Collection, University of Illinois at Urbana-Champaign, Urbana. Soybean seeds were surface sterilized by soaking in 2.1% sodium hypochlorite for 20 min followed by two min washes in sterile distilled water. Seeds then were plated onto sucrose water agar (5.0% sucr se in 0.8% agar) medium (SWA) to all w germination and t select for sterile seeds. Germinating seeds were transferred to 25- × 150-mm test tubes containing 10 ml of SWA.

Bacteria. Four strains f *A. rhizogenes* were evaluated f r their ability to induce transf rmed roots on 10 soybean geno es. Two agropine-type strains, A4 and 1855, and ne mannopine strain, 8196, were from our collection. The cucumopine strain, K599, was obtained from Allen Kerr, Waite Institute, Glen Osmond, 5064—South Australia. Nonpathogenic strain NT-1 is *A. tumefaciens* strain C58 cured of its Ti plasmid (34). Bacterial strains were grown in yeast extract-mannitol liquid medium (27) with aeration at 28 C.

Plant inoculations. Soybean seedlings were inoculated after the emergence vegetative stage (10). The onset of vegetative stage in the 10 selected soybean seedling genotypes varied between 6 and 15 days after plating seed on SWA. Inoculations were performed with a scalpel previously dipped into an overnight culture of the strain of *Agrobacterium* being tested. Cotyledons were inoculated by cutting the abaxial face several times to form a checked wound site. Hypocotyl segments were inoculated by making 2.0-cm-long longitudinal cuts. Twenty seedlings of each genotype were inoculated at each site for each bacterial strain tested. Inoculated seedlings were returned to 25- × 150-mm test tubes and incubated in growth chambers under cool-white fluorescent lighting for a 16-hr photoperiod at 25 C.

Establishment of root cultures. Cotyledons and hypocotyls with root primordia were transferred to 25 ml of liquid MonMor medium in 25- × 100-mm culture plates. MonMor medium consisted of Monnier's salts (17) containing Morel's vitamins (18), 86 mg L^{-1} of ferric-sodium salt EDTA according t Murashige and Skoog medium (21) and 20.0 g L^{-1} of sucrose. The pH was adjusted to 5.8 before autoclaving for 20 min at 118 C and 1.0 g cm^{-2}. After autoclaving, the medium was cooled to approximately 45 C and carbenicillin at 500 mg L^{-1} was added to inhibit

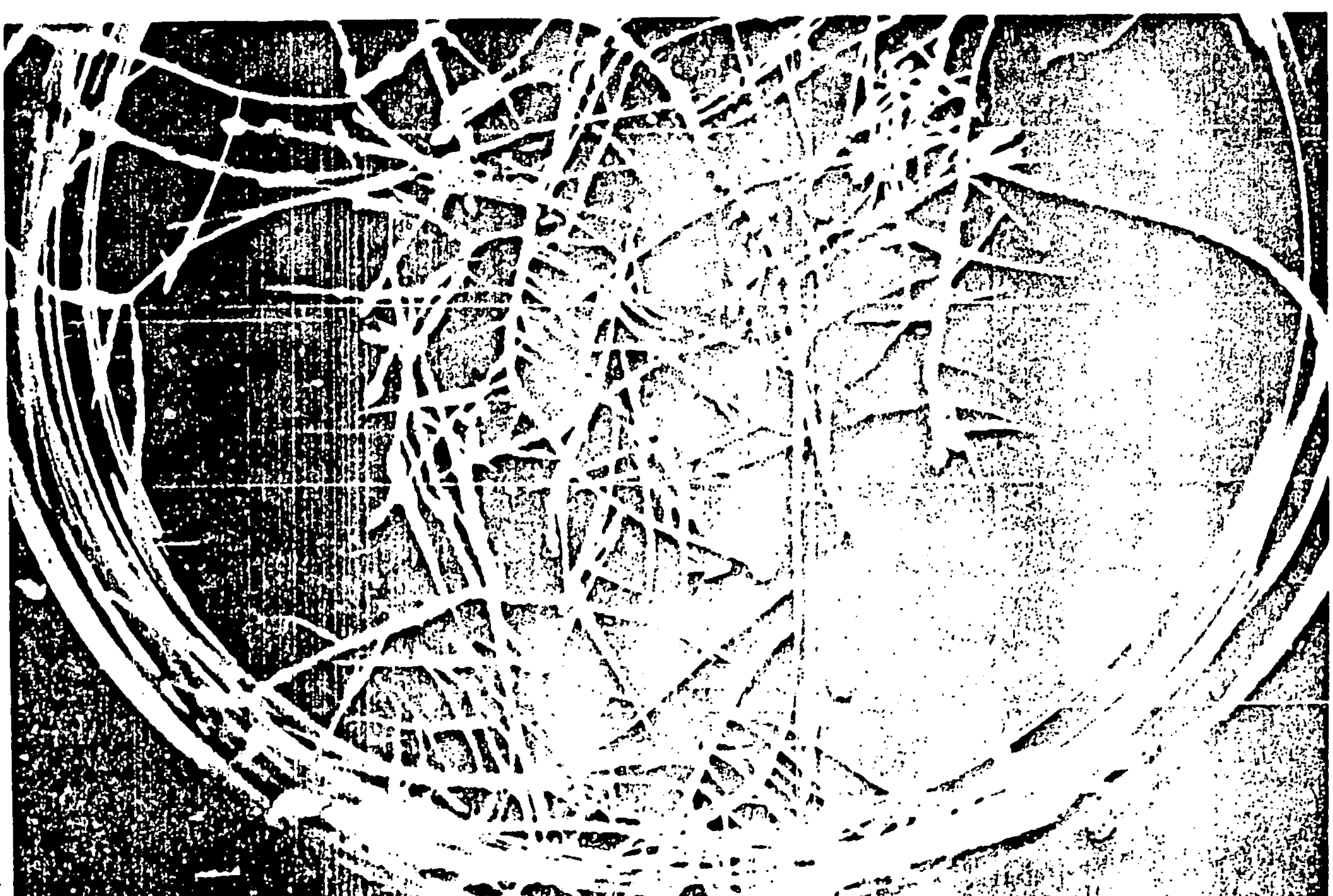

Fig. 1. Established hairy root cultures after 2 wk of growth on MonMor agar medium. Arrow indicates friable callus.

bacterial growth. For propagation of the soybean cyst nematode, approximately 2.0 g of verified transformed roots were subcultured from MonMor liquid medium to Lauritis medium (14,15) containing 16.0 g L^{-1} of Difco Bacto agar in 150- × 25-mm culture plates.

Opine analysis. For the detection of the mannityl opines, approximately 0.3 g of root tissue was macerated in 100 μl of 70% ethanol containing 10 μl of the electrophoresis running buffer (formic acid/acetic acid/water, 3:6:91, v/v/v, pH = 1.9). For detection of cucumopine, root tissue was macerated in distilled water. In each case supernatants were recovered following centrifugation. Twenty microliters of supernatant extract was spotted on Whatman 3 MM paper. The spots were allowed to dry, and the papers were wetted with the running buffer and subjected to high voltage paper electrophoresis (HVPE) at 4,000 V for 12-15 min. The electrophoretograms were dried in a stream of warm air until no odor of acetic acid could be detected.

TABLE 1. Frequency of hairy root induction on cotyledons of genotypes of *Glycine max* inoculated with one of four strains of *Agrobacterium rhizogenes*

	Opine positive roots / total roots[a] *A. rhizogenes* strain			
Genotype	K599	8196	1855	A4
Cartter	13/13[b]	0/0	1/2	0/3
Fayette	10/10	1/3	0/2	0/0
Franklin	1/1	0/0	0/0	0/0
Kent	10/10	1/3	0/0	0/0
Lee	3/3	0/0	0/0	0/0
Mandarin	17/17	1/2	0/0	1/2
Maple Arrow	15/15	2/4	1/3	1/6
Peking	1/1	0/0	0/0	0/0
Pickett	1/1	0/0	0/0	0/0
Williams 82	3/3	0/2	0/0	1/3
Total	74/74/200[c]	5/14/200	2/7/200	3/14/200

[a] In each case 20 cotyledons were inoculated with each strain of *A. rhizogenes*.
[b] Number of cotyledons yielding opine-positive roots/number of cotyledons producing roots at the wound site.
[c] Total number of cotyledons inoculated by each strain.

Mannityl opines were visualized with the alkaline silver nitrate reagents of Trevelyan and co-workers (31). Electrophoretograms were dipped in silver nitrate solution (4 g of silver nitrate in 20 ml of water diluted to 1 L with acetone) and dried thoroughly. The spots were developed by dipping in ethanolic NaOH (2% NaOH in 90% ethanol). The papers were subsequently dipped in Kodak fixer and rinsed with distilled water for 15 min (6).

Cucumopine and its acid-degradation product were visualized with the Pauly reagent by spraying the dry electrophoretograms lightly with a solution containing equal parts of sulfanylic acid (1.0% in 1 N HCl) and sodium nitrite (5.0% in water). Papers were allowed to dry and then sprayed with aqueous 15% sodium carbonate (8,24). Cucumopine and its acid-degradation product appear as reddish and bluish spots, respectively, as the paper is sprayed with sodium carbonate.

Spots were identified as opines by comparing their electrophoretic mobilities and staining properties with those of authentic standards. Mannopine, mannopinic acid, agropine, and agropinic acid were synthesized by Yves Dessaux in our laboratory. Cucumopine was syntheized from L-histidine and α-ketoglutaric acid (7) by Paul Hanselmann, also in our laboratory. Extracts prepared from normal leaf or root tissues or from authentic hairy roots of *Nicotiana tabacum* L. 'Xanthi NG' were included on electrophoretograms as negative and positive controls, respectively.

Propagation of *Heterodera glycines*. Soybean cultivar Williams 82 transformed root cultures, freshly transferred to plates containing Lauritis medium (14), were inoculated with six to eight gravid females of *H. glycines* race 3 from gnotobiotic culture (15). Alternatively, second-stage juveniles (J2) from pot cultures were collected and surface sterilized by soaking in a solution containing 100 mg L^{-1} of $HgCl_2$ and 1,000 mg L^{-1} of sterile streptomycin sulfate. Nematodes were washed twice with sterile distilled water by centifugation (16). Between 50 and 100 J2 were added to the subcultured transformed root cultures grown on Lauritis medium.

RESULTS

Differentiation of roots at inoculated sites. After approximately 10 days, globular callus tissue appeared at some of the wound sites of cotyledons inoculated with strains of *A. rhizogenes*. Extensive splitting of hypocotyls with no callus formation occurred

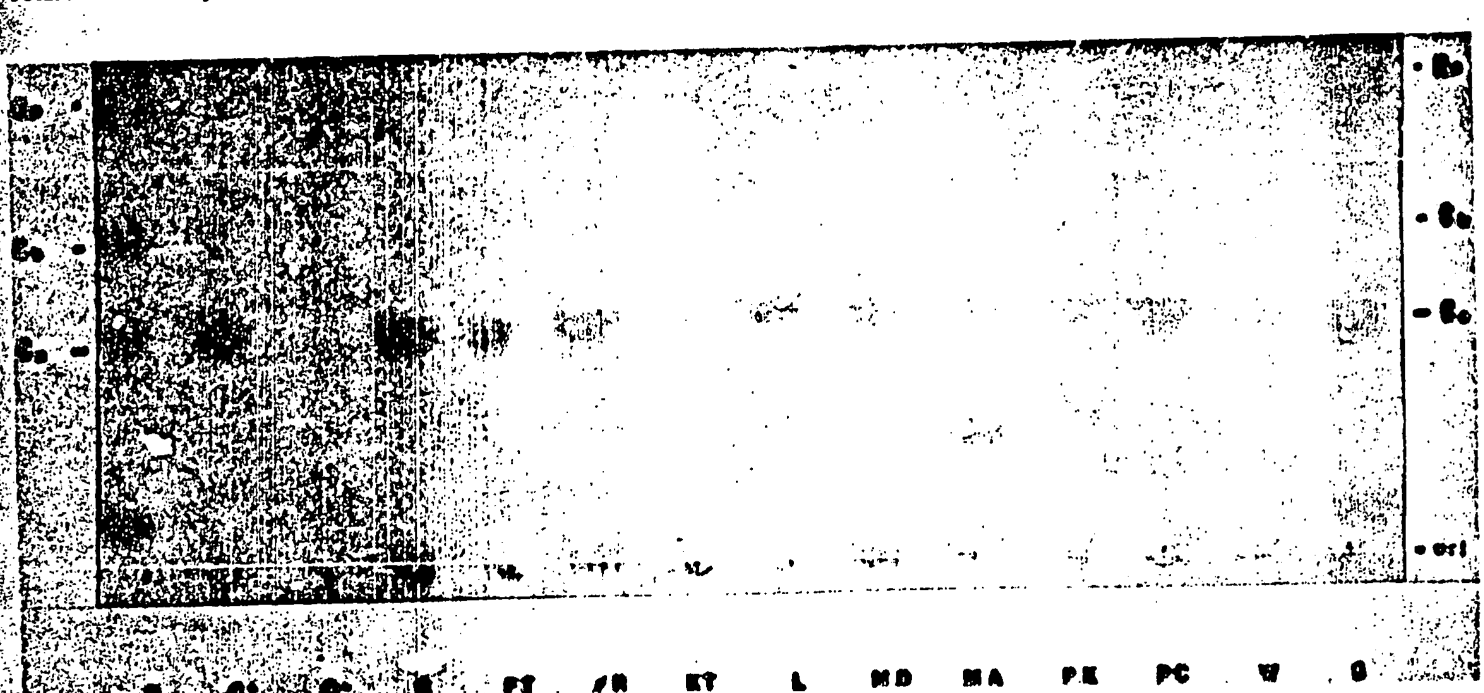

Fig. 2. Electrophoretic analysis of extracts from transformed roots of soybean incited by *Agrobacterium rhizogenes* strain K599. Standards (S) are: cucumopine (Ca), acid-degradative product of cucumopine (Cb), and histidine (Hp). (C+), Extract of tobacco hairy roots induced with cucumopine strain K599. (C-), Extract from normal tobacco roots. Other lanes contain root extracts from roots induced on genotypes: Cartter (C), Fayette (FT), Franklin (FR), Kent (KT), Lee (L), Mandarin (MD), Maple Arrow (MA), Peking (PK), Pickett (PC), and Williams 82 (W).

on inoculated hypocotyls with all bacte[illegible] strains tested. Fifteen to 25 days after inoculation of cotyledons with strains of *A. rhizogenes*, root primordia differentiated from globular callus tissue. Hypocotyls inoculated with virulent strains of *A. rhizogenes* or the nonpathogenic strain NT-1 gave rise to roots at the inoculation site and at a region about 0.5 cm below the cotyledons. Roots that developed from hypocotyls did not contain detectable opines in their cell extracts (data not shown).

When root primordia had elongated to approximately 2.0 cm, the entire hypocotyl or cotyledon was dissected from the seedling and transferred to liquid MonMor medium containing carbenicillin. Approximately 10% of the roots failed to grow in liquid MonMor medium after excision from the seedling. After 1 wk, clonal lines were established by subculturing single roots. While some subcultured roots failed to elongate, most of the roots showed growth rates of approximately 0.5 cm per 24 hr. When transferred to solid medium, many of the roots formed a small amount of friable callus at root tips (Fig. 1).

Roots containing opines were scored as being transformed (see below). Hairy root cultures were established by subculturing 4-cm segments of root meristem to 25 ml of liquid or solid MonMor medium. Hairy root cultures could be routinely maintained on solid MonMor medium by subculturing at 3-wk intervals. Hairy root cultures agitated at 60 rpm in liquid MonMor medium grew rapidly and subculturing was necessary every 10 days.

Efficiency of different strains of *A. rhizogenes*. Strain K599 was the most efficient at inciting hairy roots on cotyledons of the 10 soybean genotypes tested. This strain induced transformed roots in 5–85% of the infected cotyledons, depending on genotype (Table 1). Cucumopine, the indicator opine associated with tissues transformed by strain K599, was present in extracts from all roots tested (Fig. 2).

Root formation following inoculation with agropine strains 1855 and A4 occurred at frequencies of 3 and 7%, respectively (Table 1). However, the absence of opines in extracts indicated that most of these roots were not truly transformed (Table 1 and Fig. 3). Mannopine strain 8196 induced roots at a frequency of 7%, and only 35% of these were found to contain mannopine and mannopinic acid (Table 1 and Fig. 3).

Soybean genotypes. Efficiency of transformed root induction on cotyledons by strain K599 varied among the 10 soybean genotypes evaluated. Two genotypes, Mandarin and Maple Arrow, were quite responsive, yielding hairy roots in 75–85% of the infected cotyledons. Other genotypes, such as Franklin, Peking and Pickett, were relatively insensitive, showing infection rates of less than 10%.

Propagation [illegible] *glycines* race 3. Twenty to 25 days after inoculation (DAI) with gravid females and 16–20 DAI with J2, imbedded and emerging females were observed on Williams 82 hairy roots induced by strain K599 (Fig. 4A). Approximately 4–6 days after cyst emergence, first molting was observed followed by egg hatch and emergence of J2 (Fig 4B). Second-stage juveniles were observed migrating throughout the culture (Fig. 4C) and mature second-generation females were observed approximately 6 wk after inoculation (Fig. 4D). The nematode could be serially propagated by transferring infected hairy root segments to a fresh hairy root culture (data not shown).

DISCUSSION

The three variables tested, host genotype, strain of *A. rhizogenes*, and site of inoculation all proved important in the successful induction of hairy roots on soybeans. In general, cotyledon inoculations were more effective than stem or hypocotyl infections. This contrasts with results reported by Owens and Cress (23) who showed that stem inoculations were more effective than cotyledon infections. However, they did not characterize roots appearing at inoculation sites. Our observations that hypocotyl wound sites give rise to normal adventitious roots raises the question as to whether the roots appearing at their infection sites were truly transformed. In fact, our observations suggest that the genotypes of *G. max* tested have a propensity to form adventitious roots when inoculated with strains of *Agrobacterium*. This response depends on inoculation of *Agrobacterium* but does not require an Ri plasmid. Hypocotyl infections with strain NT-1 regularly gave rise to root proliferation at the wound sites and at a nonwounded collar region just below the cotyledons. Such roots from plants infected by *A. rhizogenes* and from plants infected with strain NT-1 contained no detectable opines. A few adventitious roots also developed from inoculated cotyledons. However, in such infections the nontransformed roots generally arose at the junction between the cotyledon and its petiole, distant from the actual wound sites. The roots forming at the wound site usually were transformed as judged by the presence of the marker opines.

Hairy root induction depended on the strain of *A. rhizogenes*. Strain K599 was by far the most effective in inducing hairy roots, with all soybean genotypes tested being sensitive to infection by this strain. The one mannopine-type and the two agropine-type strains of *A. rhizogenes* tested were much less effective at inducing hairy roots on soybeans (Table 1). These results are consistent with those of Byrne and co-workers (2) who failed to observe

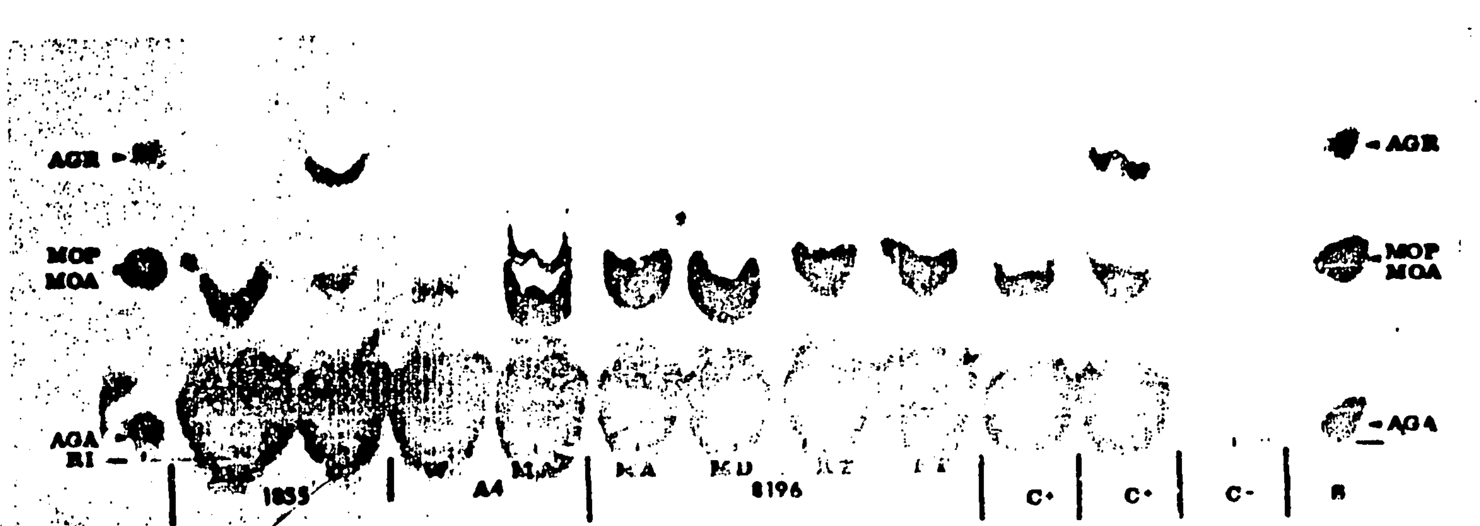

Fig. 3. Electrophoretic analysis of extracts from transformed roots incited by mannopine and agropine-type *Agrobacterium rhizogenes* strains. Standards (S) are: agropine (AGR), mannopine (MOP), mannopinic acid (MOA), and agropinic acid (AGA). Mannopine and mannopinic acid comigrate under these electrophoretic conditions. Other lanes contain root extracts from: Maple Arrow (MA) and Cartter (C) induced by strain 1855; Williams 82 (W) and Maple Arrow (MA) induced by strain A4; Maple Arrow (MA), Mandarin (MD), Kent (KT) and Fayette (FT) induced by strain 8196. (C+), Extracts from tobacco hairy roots incited by strains 8196 and 1855. (C−), Extracts from normal tobacco roots.

any hairy r ot induction on 17 genotypes of *G. max* by a strain of *Agrobacterium* containing pRi8196. Nor did strain 8196 induce hairy roots on *G. soja* r *G. canescens*. This is consistent with our bservati n that strain 8196 shows p or hairy r ot induction on the genotypes f *G. max* we tested (Table 1). However, our results contrast with experiments reported by Pech et al (28) on transformati n f ther *Glycine* spp. They bserved that, alth ugh frequencies varied, a strain harb ring the agropine-type Ri plasmid, pRi1855, was highly effective in transforming several accessions f *G. canescens*, *G. clandestina*, and *G. argyrea*. They also found hyp cotyls to be more responsive than cotyledons. These differences may be due to dissimilarities in host plant species, chromosomal backgrounds of the bacteria, cultural conditions, or a combination of the three factors.

Hairy root formation also depended on the host plant genotype. Based on frequencies at which opine-positive roots arose, the 10 soybean genotypes tested could be divided into two groups. Genotypes Cartter, Fayette, Kent, Mandarin, and Maple Arrow were judged t be sensitive, showing frequencies of hairy root formation by strain K599 ranging from 50 to 85%. The remaining gen types were relatively insensitive with transformation frequencies by this strain below 20%. Although the numbers are low, the few productive infections with the agropine- and mannopine-type strains of *A. rhizogenes* occurred most frequently on those gen types susceptible to infection by strain K599 (Table 1).

Roots at wound sites were judged as transformed if opines were detected in cell-free extracts. Such opine-positive roots generally exhibited other phenotypes associated with true hairy r ots including fast growth in culture, loss f geotropism, and lateral root branching (Fig. 1; 22,30). N morphol gical differences were noted among opine-positive roots of various *G. max* genotypes. When established in tissue culture, opine-positive hairy roots retained their transformed phenotypes. Furtherm re, axenic root cultures c uld be maintained f r at least 1 yr by transferring root tip cuttings fr m older cultures t fresh medium.

While identification based on opine content is sound for analysis of roots induced by the cucumopine and mannopine strains, it may underestimate the frequency f transformation by agr pine strains. This is because, unlike cucumopine and mann pine strains, the opine biosynthetic genes in the agropine-type Ri plasmids are encoded on a T-DNA segment separate from that which encodes the *onc* genes (9,36). Thus, it is possible that s me of the roots resulting from infection by the agropine strains were transformed but contained only the oncogenic T-DNA segment (3,4). However, the two agropine strains tested were inefficient at inducing either adventitious or transformed roots at w und sites (Table 1).

Hairy root cultures of Williams 82 inoculated with *H. glycines* race 3 produced mature cy ts approximately 21 days after nematode inoculation (Fig. 4B). Root cultures could be infected with gravid females or with J2, although inoculation with the f rm r was simpler and appeared to be more efficient. The time required for development of mature cysts was similar to that reported for *H. glycines* on axenic explant cultures of normal soybean

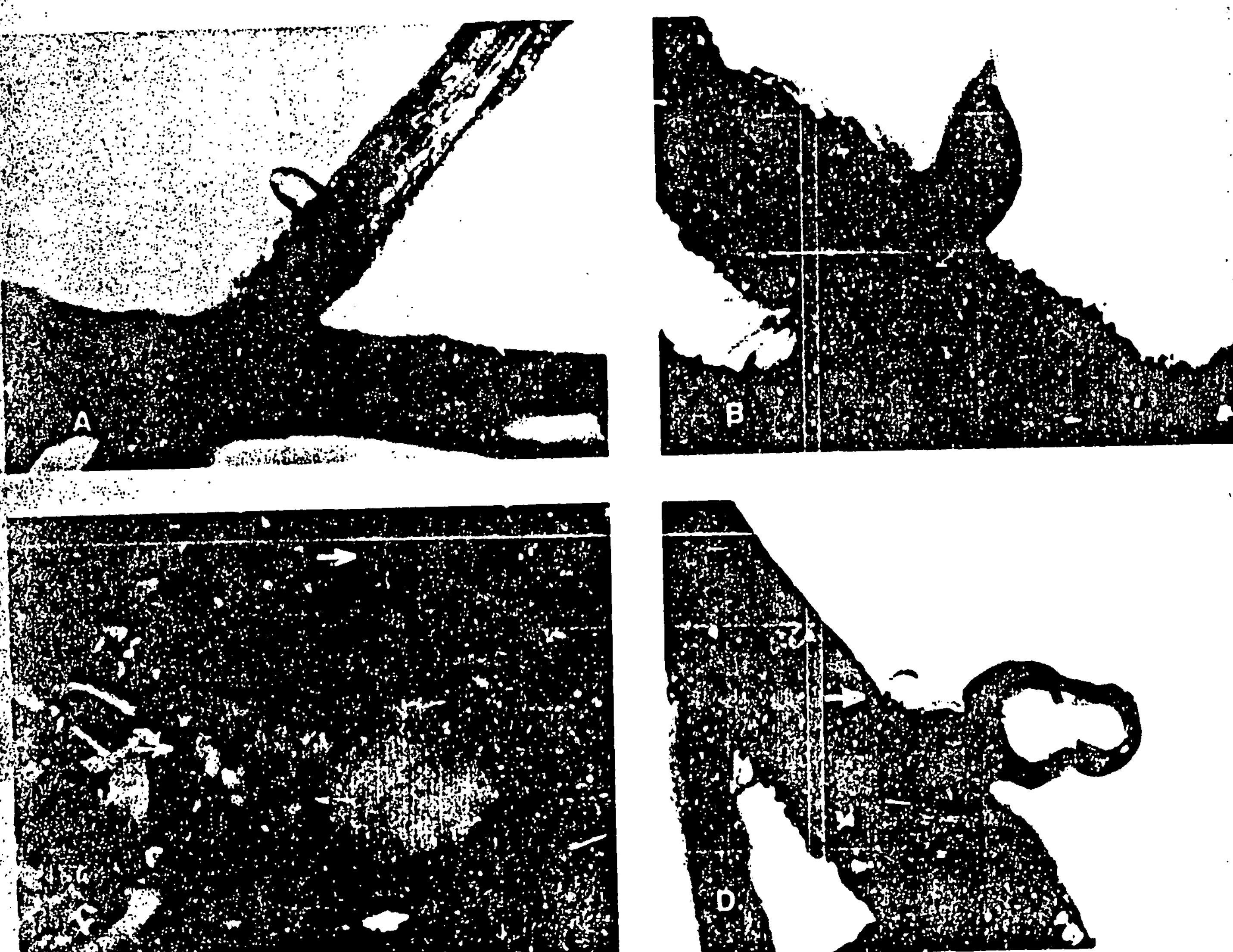

Fig. 4. Propagati n f *Heterodera glycines* n transformed roots of soybean cultivar Williams 82. A, Female feeding 14 days after inoculation with second-stage juveniles. B, Female 21 days after inoculation. C, Emergence of second-stage juvenile from cyst and juvenile (top arrow) probing root surface. D, Migrating juvenile (arr w).

roots (14,15). After an additi nal 3 wk, second-generation cysts were bserved, indicating that the ne de could complete its entire life cycle in transf rmed root cultures (Fig. 4A).

Hairy root cultures may provide some advantages ver normal root explants for monoxenic culture f *H. glycines*. First, transformed roots grow indefinitely in tissue culture bviating the need t periodically reestablish new root explants from germinating seedlings. Furtherm re, because the transf rmed roots are cl nal in origin, established hairy root cultures should assure a unif rmity in genetic background. Second, hairy root cultures may enhance reproductive capacity of the nematode. Such was the case f r the propagation of *M. javanica* on cultured tomato hairy roots (33). This increase in reproduction was ascribed to the large numbers of lateral roots produced by the transformed tissues (33). Root branching also is characteristic of soybean hairy root cultures (Fig. 1). Third, since the *A. rhizogenes* system provides a way t insert new genes into differentiated tissues, novel genes conferring nematode resistance or the biosynthesis of potential control compounds could be engineered into the soybean genome and directly tested for their efficacy in conferring resistance to *H. glycines*. Finally, a simple method to axenically cultivate the soybean cyst nematode could be of considerable value in the study of the molecular biology and genetics of *H. glycines*.

LITERATURE CITED

1. Binns, A. N., and Thomashow, M. F. 1988. Cell biology of *Agrobacterium* infection and transformation of plants. Annu. Rev. Microbiol. 42:575-606.
2. Byrne, M. C., McDonnell, R. E., Wright, M. W., and Carnes, M. G. 1987. Strain and cultivar specificity in the *Agrobacterium*-soybean interaction. Plant Cell Tissue Organ Cult. 8:3-15.
3. Cardarelli, M., Mariotti, D., Pomponi, M., Spano, L., Capone, I., and Costantino, P. 1987. *Agrobacterium rhizogenes* T-DNA genes capable of inducing hairy root phenotype. Mol. Gen. Genet. 209:475-480.
4. Cardarelli, M., Spano, L., De Paolis, A., Mauro, M. L., Vitali, G., and Costantino, P. 1985. Identification of the genetic locus responsible for non-polar root induction by *Agrobacterium rizogenes* 1855. Plant Mol. Biol. 5:385-391.
5. Chilton, M-D., Tepfer, D. A., Petit, A., David, C., Casse-Delbart, F., and Tempé, J. 1982. *Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host plant root cells. Nature (London) 295:432-434.
6. Dahl, G. A., Guyon, P., Petit, A., and Tempé, J. 1983. Silver nitrate-positive opines in crown gall tumors. Plant Sci. Lett. 32:193-203.
7. Davioud, E., Quirion, J-C., Tate, M. E., Tempé, J., and Husson, H-P. 1988. Structure and synthesis of cucumopine, a new crown gall and hairy root opine. Heterocycles 27:2423-2430.
8. Dawson, R. M. C., Elliot, D. C., Elliot, W. H., and Jones, K. M. 1968. Page 530 in: Data for Biochemical Research, 2nd ed. Clarendon Press, Oxford.
9. De Paolis, A., Mauro, M. L., Pomponi, M., Cardarelli, M., Spano, L., and Costantino, P. 1985. Localization of agropine synthesizing functions in the TR-region of the root inducing plasmid of *Agrobacterium rhizogenes* 1855. Plasmid 13:1-7.
10. Fehr, W. R., and Caviness, C. E. 1977. Stages of soybean development. Coop. Ext. Serv., Iowa State Univ., Ames. Special Rep. 80:1-12.
11. Filetici, P., Spano, L., and Costantino, P. 1987. Conserved regions in the T-DNA of different *Agrobacterium rhizogenes* root-inducing plasmids. Plant Mol. Biol. 9:19-26.
12. Ichinohe, N. 1961. Studies on the soybean cyst nematode, *Heterodera glycines*. Hokkaido Nat. Exp. Stn. Rep. 55:1-77.
13. Isogai, A., Fukuchi, N., Hayashi, M., Kamada, H., Harada, H., and Suzuki, A. 1988. Structure of a new opine-mikimopine in hairy root induced by *Agrobacterium rhizogenes*. Agric. Biol. Chem. 52:3235-3238.
14. Lauritis, J. A., Rebois, R. V., and Graney, L. S. 1982. Technique for gnotobiotic cultivation of *Heterodera glycines* Ichinohe n *Glycine max* (L.) Merr. J. N ol. 14:122-124.
15. Lauritis, J. A., Reb . V., and Graney, L. S. 1983. Development f *Heterodera glycine* Ichin he on soybean, *Glycine max* (L.) Merr. under gnotobiotic conditions. J. Nematol. 15:272-281.
16. Lawn, D. A., and Noel, G. R. 1986. Gnotobiotic culture of *Pratylenchus scribneri* on carrot discs. Nematropica 16:45-51.
17. Monnier, M. 1976. Culture in vitro de l'embryon immature de *Capsella bursa pastoris*. Moench Rev. Cytol. Bi l. Veg. 39:1-9.
18. Morel, G., and Wetmore, R. H. 1951. Fern callus tissue cultur Am. J. Bot. 38:141-143.
19. Mugnier, J. 1987. Infection of *Polymyxa betae* and *Plasmodiophora brassicae* n roots containing root-inducing transferred DNA f *Agrobacterium rhizogenes*. Phytopathology 77:539-542.
20. Mugnier, J., and Mosse, B. 1987. Vesicular-arbuscular mycorrhizal infection in transformed root-inducing T-DNA roots grown axenically. Phytopathology 77:1045-1050.
21. Murashige, T., and Skoog, F. 1962. A revised medium f r rapid growth and bioassay with tobacco tissue cultures. Physi l. Plant 15:473-494.
22. Ooms, G., Karp, A., Burrell, M. M., Twell, D., and R berts, J. 1985. Genetic modification of potato development using Ri T-DNA. Theor. Appl. Genet. 70:440-446.
23. Owens, L. D., and Cress, D. E. 1985. Genotypic variability of soybean response to *Agrobacterium* strains harboring the Ti or Ri plasmids. Plant Physiol. 77:87-94.
24. Petit, A., Berkaloff, A., and Tempé, J. 1986. Multiple transformation of plant cells by *Agrobacterium* may be responsible for the complex organization of T-DNA in crown gall and hairy root. M l. Gen. Genet. 202:388-393.
25. Petit, A., David, C., Dahl, G., Ellis, J. G., Guyon, P., Casse-Delbart, F. C., and Tempé, J. 1983. Further extension of the pine concept: Plasmids in *Agrobacterium rhizogenes* cooperate f r opine degradation. Mol. Gen. Genet. 19:204-214.
26. Petit, A., and Tempé, J. 1985. The function of T-DNA in nature. Pages 625-636 in: Molecular Form and Function of the Plant Genome. L. Van Vloten-Doting, G. S. P. Groot, and T. G. Hall, eds. Plenum Publishing Co., New York.
27. Petit, A., Tempé, J., Holsters, M., Van Nontagu, M., and Schell, J. 1978. Substrate induction of conjugative activity of *Agrobacterium tumefaciens* Ti plasmids. Nature (London) 271:570-571.
28. Rech, E. L., Golds, T. J., Hammatt, N., Mulligan, B. J., and Davey, M. R. 1988. *Agrobacterium rhizogenes* mediated transformati n of the wild soybeans *Glycine canescens* and *G. clandestina*: Production of transgenic plants of *G. canescens*. J. Exp. Bot. 39:1275-1285.
29. Riggs, R. D., and Schmitt, D. P. 1989. Soybean cyst nematode. Pages 65-67 in: Compendium of Soybean Diseases, 3rd ed. J. B. Sinclair and P. A. Backman, eds. The American Phytopathological Society, St. Paul, MN.
30. Tepfer, D. 1984. Transformation of several species of higher plants by *Agrobacterium rhizogenes*: Sexual transmission of the transformed genotype and phenotype. Cell 37:959-967.
31. Trevelyan, W. E., Procter, D. P., and Harrison, J. P. 1950. Detection of sugars on paper chromatograms. Nature (London) 166:444-445.
32. Verdejo, S., and Jaffe, B. A. 1988. Reproduction of *Pasteuria penetrans* in a tissue-culture system containing *Meloidogyne javanica* and *Agrobacterium rhizogenes*-transformed roots. Phyt path logy 78:1284-1286.
33. Verdejo, S., Jaffee, A., and Mankau, R. 1988. Reproducti n f *Meloidogyne javanica* on plant roots genetically transformed by *Agrobacterium rhizogenes*. J. Nematol. 20:599-604.
34. Watson, B., Currier, T. C., Gordon, M. P., Chilton M-D., and Nester, E. W. 1975. Plasmid required for virulence of *Agrobacterium tumefaciens*. J. Bacteriol. 123:255-264.
35. White, F. F., and Nester, E. W. 1980. Hairy root: Plasmid encodes virulence traits in *Agrobacterium rhizogenes*. J. Bacteriol. 141:1134-1141.
36. White, F. F., Taylor, B. H., Huffman, G. A., Gordon, M. P., and Nester, E. W. 1985. Molecular and genetic analysis of the transferred DNA regions of the root-inducing plasmid of *Agrobacterium rhizogenes*. J. Bacteriol. 164:33-44.
37. Zambryski, P., Tempé, J., and Schell, J. 1989. Transfer and functi n of T-DNA genes from *Agrobacterium* Ti and Ri plasmids in plants. Cell 56:193-201.